Laboratory Examinations of Transmissible Spongiform Encephalopathies in Denmark during 2013

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Report

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Report from the National Veterinary Institute, Technical University of Denmark
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Introduction

The aim of this report is to give detailed information on the diagnostic examination on transmissible spongiform encephalopathies (TSE) performed in Denmark during 2013. The present annual report is the 18th on this topic published by the National Veterinary Institute, Technical University of Denmark (DTU-VET).

The report is made to fulfil the demands given by the EU Commission (Regulation No 999/2001 of the European Parliament and the Council of 22. May 2001) and the Office International des Epizooties (OIE) (Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th edition 2008, Chapter 2.4.6 and Chapter 2.7.13) regarding diagnostic examinations.

The DTU-VET is the national reference laboratory of bovine spongiform encephalopathy (BSE) and TSE/Scrapie, and therefore the results of all neuropathological examinations on BSE and Scrapie in Denmark are given in the present report as in previous years.

Assignments

This year's report includes all examinations of adult cattle, sheep, and goats displaying behaviour disorders and/or neurological signs with suspicion of having TSE as well as animals with neurological signs but without suspicion of TSE. Furthermore, the report includes examinations of pet animals, fur bearing animals, wild animals, and zoo mammals displaying behaviour disorders and/or neurological signs. The number of pet animals, wildlife, and zoo animals examined, however, do not necessarily include all examined animals in Denmark since neuropathological examination may have been performed at other laboratories without the knowledge of the DTU-VET. In no case, however, suspicion of TSE was made. Similar, neuropathological examination of adult ruminants without clinical signs or having diseases not compatible with BSE or Scrapie may have been performed at other institutions. However, the brainstems of these animals were examined according to the surveillance program described below.

The Danish BSE surveillance program included during 2013 the testing of the following bovine animals:

- Bovines with a clinical suspicion of BSE. All cases examined at DTU-VET
- Emergency slaughter bovines older than 48 months. Private, approved laboratories
- Bovines older than 48 months with remarks at the ante mortem inspection performed by the official veterinarian at the slaughterhouse. Private, approved laboratories
- All fallen stock of bovines older than 48 months. Private, approved laboratory and DTU-VET.
- A random sample of the bovines for human consumption older than 72 months from 1 January 2013. From 3 July no testing of healthy slaughter bovines Private, approved laboratories.

The Danish TSE surveillance program included during 2013 the testing of the following small ruminants:

- Small ruminants with a clinical suspicion of TSE. All cases examined at DTU-VET
- A random sample of fallen stock animals older than 18 months so we fulfil the requirements of the TSE legislation, which with the Danish sheep and goat population in 2013 was annually 500 sheep tests and 100 goat tests. Private, approved laboratories.

DTU-VET carried all tests in agreement with Annex X in the Regulation (EC) No. 999/2001.

DTU-VET controlled test performance in the private laboratories. This included weekly submission of control data for review and an annual inspection visit.
The laboratories' approval of daily set-up's are done according to specified criteria, incorporating results of positive and negative controls in IDEXX HerdCheck and PrioSTRIP assays. Assignment of test-positivity, test-negativity of tested tissue samples is made in relation to prefixed criteria as approved by the national reference laboratory. Retesting and possible reclassification of single samples with a positive or doubtful result takes place according to a prefixed schedule, in the case of borderline reactions or by demonstrable technical errors, such as grossly differing readings of duplicates or appearance of "clusters" of positive microtiter wells. Proficiency trials were performed twice a year co-ordinated by AHVLA.

Staff
One veterinary pathologist trained in the diagnosis of TSE at the EU Community Reference laboratory for TSE, AHVLA, Weybridge, UK, performed all the neurohistopathological examinations and IHC in Copenhagen.
In the Section for Public sector service and commercial diagnostics, another veterinarian served the rapid tests/screening analyses whereas one senior advisor performed the supervision of private, rapid testing laboratories. Furthermore, the TSE genotyping is performed by a molecular biologist.

Methods

Methodology for examination of clinically suspected cases of BSE or TSE/Scrapie

Clinically suspected cases of BSE or TSE/Scrapie were usually euthanized by intravenous injection of an overdose of barbiturate, and the heads were submitted - generally overnight - to DTU-VET. The whole brain was removed from the skull. A cross-sectional sample of 5-10 gram from the brainstem just caudal to the obex region was taken and used for IDEXX HerdCheck according to the manufacture guidelines (the remaining material was kept at 5ºC until the case was completed).

The rest of the brainstem was fixed in 10% neutral buffered formalin for 3 days. Brainstem areas were selected according to the OIE Manual (medulla at the obex, medulla through the caudal cerebellar peduncles and midbrain through rostral colliculi), cut into 3-5 mm tissue blocks and post fixed for a few days with daily change of formalin.

One half of the cerebrum was kept at 5ºC until the case was completed. The rest of the brain material was fixed in 10% neutral buffered formalin for two weeks with change of fixative after one week. Transverse sections of the cerebrum and longitudinal sections of the cerebellum and the pituitary were, cut into 3-5 mm tissue blocks and post fixed for a few days with daily change of formalin.

For histopathology, sections were stained with haematoxylin and eosin. Furthermore, immunohistochemistry (IHC) for demonstration of disease specific prion protein (PrP^D) was applied on the obex section of all cases.

If a case was inconclusive material was submitted to the EU Community Reference Laboratory for TSE, Weybridge, UK, for further examination.

In case of positive cases, fresh brainstem material was furthermore analysed by AFSSA Discriminatory Western Blotting for PrP classification.

Methodology for examination of animals-at-risk (fallen stock, etc.)

At DTU-VET the testing is performed according to the following scheme:

The brainstem was removed on location (by the foramen magnum technique) and submitted to the DTU-VET in suitable containers (cooled or at room temperature and without any fixative).

The first part of the testing was by IDEXX HerdCheck as the monitoring test, taking a 0.3 gram sample from one half of the obex region. The remaining brain stem with the intact other half of the obex region was stored refrigerated for confirmatory testing in case of a positive or inconclusive result.

If IDEXX HerdCheck produced a negative result, the specimen was reported as negative without further examination. In case of a positive result, samples of the remaining brainstem were subjected to confirmatory testing by AFSSA Discriminatory Western Blotting for PrP classification, histopathology and IHC. The case was reported as BSE/Scrapie-positive if confirmatory testing revealed a positive result.

In cases of severely autolysed / unsuitable material, that was IDEXX HerdCheck positive tissue was forwarded to the EU Community Reference laboratory for TSE, AHVLA, Weybridge, UK., for further examination.

Methodology for confirmatory examination of animals TSE positive by monitoring testing (when the rapid testing are positive)

In case of a positive or inconclusive result of the monitoring tests (rapid test) the confirmatory examinations were performed at DTU-VET according to the following procedure:
A cross-sectional sample of 5-10 gram from the brainstem just caudal to the obex region was taken and used for the IDEXX HerdCheck analysis, as described above, if the monitoring test was performed at a private laboratory. In case of IDEXX HerdCheck positive cases a sample of brainstem was furthermore analysed in the AFSSA Discriminatory Western Blotting.

For additional confirmatory examination the brainstem was fixed in 10% neutral buffered formalin for 3 days. Brainstem areas were selected according to the OIE Manual (medulla at the obex (and medulla through the caudal cerebellar peduncles and midbrain through rostral colliculi if possible). Cut into 3-5 mm tissue blocks (obex), and post fixed for a few days with daily change of formalin. Sections were stained with haematoxylin and eosin as well as by immunohistochemistry (IHC) for demonstration of disease specific prion protein (PrP^D).

The case was reported as BSE/Scrapie-positive if confirmatory testing revealed a positive result. In cases of severely autolysed / unsuitable material that was negative in AFSSA Western Blotting tissue was forwarded to the EU Community Reference laboratory for TSE, AHVLA, Weybridge, UK, for further examination.

Methodology for examination of wildlife animals, pet animals, fur animals, zoo, and other animals

Mink older than seven months of age and other animals older than 1 year of age with clinical signs indicating a neurological disorder were examined for spongiform encephalopathies. Furthermore, fallen, adult animals with CNS lesions are reported. Only mammalian species were included.
The brain was divided into two equal parts by longitudinal section. One half of the brain was fixed in 10% neutral buffered formalin for two weeks. The fixative was changed after one week. The other half was stored at - 18°C. The formalin fixed tissue was cut with 4 transversal sections into 5 equally large portions (ensuring that brain stem areas were selected according to the OIE Manual medulla at the obex, medulla through the caudal cerebellar peduncles and midbrain through rostral colliculi) and embedded in paraffin in a routine manner. Transverse sections of cerebellum were always included. Sections were stained with haematoxylin and eosin and examined histopathologically including spongiform encephalopathies.

Results

BSE in bovines born in Denmark
During 2013 no case of indigenous BSE was diagnosed.

Scrapie in small ruminants born in Denmark
During 2013 no case of indigenous TSE was diagnosed.

Examination of suspected BSE and Scrapie cases
During 2013 two suspicions of clinical TSE in bovines were reported – both were PrP^D negative. No suspicion in small ruminants. Table 1 and 2.

In addition, from an outbreak of clinical listeriosis among milking goats one goat (ID 25973-2290 from CHR 114891) was examined histopathologically revealing typical signs of listeriosis with multifocal non-suppurative to necrotizing encephalitis. The goat was not PrP^D tested due to the anamneses involving several animals and the acute disease.

Examination of animals-at-risk (fallen stock, etc.)
This year 20.133 bovines have been tested including 1100 cases analysed at DTU-VET, Table 3. The remaining animals were analysed at private laboratories.

Moreover, 637 sheep and goats have been tested negative for TSE, all analysed at private laboratories.

Examination of healthy slaughter animals, confirmatory testing
The private, approved laboratories tested 3.342 slaughter animals during 2013. The laboratories found no animals positive.

Examination of wildlife animals, pet animals, fur animals, and zoo animals
Neither lesions consistent with chronic wasting disease nor other CNS lesions were observed in adult fissipeds and no other examined adult animals showed CNS lesions.

Table 1. Cause of submission for suspected cases of bovine spongiform encephalopathy and Scrapie during 2013.
According to Order no. 800 of 13. July 2006 and 930 of 7 September 2006 on Bovine Spongiform Encephalopathy and Scrapie.

Cause	**Numbers**
Animals displaying behaviour disorders and/or neurological signs	2
Moribund animals without signs of infectious disease or traumatic signs	0
Other progressive diseases	0
No available information	0
Non-TSE signs	
Acute unset of neurological signs among several animals, suspicion of listeriosis	0

Table 2. Summary of neuropathological findings in two cattle suspected of having transmissible spongiform encephalopathy 2013.

Run. No.	Date	Lab. No.	CHR. No.	CKR.No.	Breed	Age (months)	Region	Histopathology	Additional results regarding BSE	Year of confirmation
1	06-02-2013	2013-10-261	59446	59446-2572	Cow	60	North	Pituitary gland abscess	Negative by IHC and IDEXX Herdcheck	2013
2	12-11-2013	2013-10-2386	71218	71218-2410	Cow	55	East	Lesions consistent with lister-iosis	Negative by IHC and IDEXX Herdcheck	2013

Table 3. Laboratory examinations at the DTU-VET 2013.
In accordance with the Danish surveillance programme and other tests.

Bovines. Group of animals	Samples	Negative	Positive
Emergency slaughter animals	**0**	**0**	**0**
Remarks at the ante mortem inspection	**0**	**0**	**0**
Fallen stock	**1100**	**1100**	**0**
Subtotal	**1100**	**1100**	**0**
Clinical suspects	**2**	**2**	**0**
Confirmatory testing of slaughter animals	**0**	**0**	**0**
Confirmatory testing of fallen stock	**0**	**0**	**0**
Total, bovines	**1102**	**1102**	**0**
Small ruminants. Group of animals			
Clinical suspects	**0**	**0**	**0**
Confirmatory testing of slaughter animals	**0**	**0**	**0**
Confirmatory testing of fallen stock	**0**	**0**	**0**
Fallen stock	**0**	**0**	**0**
Total, small ruminants	**0**	**0**	**0**
Wildlife and other animals etc.	**0**	**0**	**0**